Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

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To cite this article: Jong-Hee Ko, Hyuk-Sang Kwon, Jong-Min Yoon, Jong-Su Yoo, Hyeon-Soo Jang, Ji-Young Kim, Seung-Woo Yeon & Jae-Hoon Kang (2015) Effects of *Polygonatum sibiricum* rhizome ethanol extract in high-fat diet-fed mice, Pharmaceutical Biology, 53:4, 563-570, DOI: 10.3109/13880209.2014.932393

To link to this article: https://doi.org/10.3109/13880209.2014.932393

Published online: 20 Oct 2014.

Pharmaceutical Biology

http://informahealthcare.com/phb
ISSN 1388-0209 print/ISSN 1744-5116 online
Editor-in-Chief: John M. Pezzuto
Pharm Biol, 2015; 53(4): 563–570
 DOI: 10.3109/13880209.2014.932393

informa
healthcare

ORIGINAL ARTICLE

Effects of *Polygonatum sibiricum* rhizome ethanol extract in high-fat diet-fed mice

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Abstract

Context: The rhizome of *Polygonatum sibiricum* Redoute (Liliaceae) has long been used to treat diabetes-associated complications. However, the pharmacological mechanism of *P. sibiricum* on metabolic disorders is not clear.

Objective: This study investigates the effect of an ethanol extract of *P. sibiricum* rhizomes (designated ID1216) on obesity conditions including weight loss in high-fat (HF) diet-fed mice and explores the potential underlying mechanisms.

Methods: To identify the metabolic impact of the *P. sibiricum* rhizome extract, HF diet-fed mice were administered ID1216 orally at doses of 250 and 1000 mg/kg/d for 10 weeks, and various factors related to metabolic syndrome were analyzed. We also examined the effects of ID1216 on the expression of genes involved in adipogenesis and lipolysis in 3T3-L1 cells, as well as genes associated with energy homeostasis in C2C12 myocytes.

Results: ID1216 administration led to significant decreases in body weight gain (37.5%), lipid accumulation in adipose tissues (52.8%), and the levels of plasma triglycerides (26.4%) and free fatty acids (28.1%) at a dose of 250 mg/kg/d, compared with the vehicle-treated group, as well as improved insulin resistance. In addition, ID1216 was found to regulate the expression of genes related to adipogenesis and fatty acid oxidation in 3T3-L1 cells and enhance the expression of genes that modulate energy homeostasis in C2C12 myocytes.

Conclusions: ID1216 may be a promising therapeutic agent for improving obesity conditions through the sirtuin-1 and peroxisome proliferator-activated receptor γ coactivator-1α pathway.

Keywords

Metabolic disorder, Obesity, SIRT1

History

Received 27 August 2013
Revised 13 May 2014
Accepted 4 June 2014
Published online 20 October 2014

Introduction

Obesity is one of the largest public health issues in many countries and it is rapidly increasing worldwide. It is the main cause of many disorders, including insulin resistance, fatty liver, cardiovascular diseases, hypertension, infertility, cardiac hypertrophy, renal failure, cancers, and decreased life span (Abel et al., 2008; Hall et al., 2014; Jakobsen et al., 2007; Sallmen et al., 2006). Excess accumulation of visceral abdominal fat appears to be a key feature of abdominal obesity and contributes to the development of metabolic syndrome (Lim, 2014; Shimabukuro et al., 2013). Therefore, preventing abdominal fat accumulation is an ideal option for treating obesity and related diseases. Sibutramine and orlistat are the most frequently used drugs for treating obesity; however, the Food and Drug Administration (FDA) recently withdrew sibutramine from the market in light of clinical trial data indicating an increased risk for stroke and myocardial infarction. The FDA also approved a revised label for orlistat to include new safety information regarding a few cases of severe liver injury (Colman, 2012). Currently, new therapies with improved efficacy and safety are urgently needed to reduce body weight.

Polygonatum spp. (Liliaceae) are widely distributed in China, Japan, and Korea. Among the 40 species and three varieties known worldwide, *Polygonatum falcatum* A. Gray, *Polygonatum odoratum* (Mill.) Druce, and *Polygonatum sibiricum* Redoute are the representative species. The dried rhizome of *P. sibiricum* has been used in folk medicine to treat diabetes, and its efficacy and safety have been proven by many years of empirical use (Wujisguleng et al., 2012). Several studies have reported that the rhizome of *P. sibiricum* has hypoglycemic activity *in vivo* (Kato & Miura, 1993) and that a methanol extract can decrease blood glucose levels by down-regulating the glucose transporter 2 expression in the total membrane of the rat liver (Kato et al., 1994; Miura & Kato, 1995; Miura et al., 1995; Shu et al., 2012). In addition, the dried rhizome of *P. sibiricum* was reported to have multiple pharmacological activities, including antiaging effects, the ability to improve metabolic syndrome, anti-infection effects (Chen & Li, 1993), a protective effect against β-amyloid (1–42) insults in rat neuronal cells (Kim et al., 2007) and a potent antioxidant activity (Debnath et al., 2013).

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Because the effect of *P. sibiricum* on metabolic disorders has not been fully evaluated, more in-depth studies are required.

Therefore, we investigated the metabolic effect of an 80% ethanol extract of the dried *P. sibiricum* rhizome (designated ID1216) in high-fat (HF) diet-fed mice. We focused on the expression of genes that regulate adipogenesis and thermogenesis, which are known to be closely related to the etiologies of obesity and obesity-related metabolic disorders (Puigserver et al., 1998; Spiegelman et al., 1993). To reveal underlying mechanisms at the molecular level, we further examined the effects of ID1216 on the expression of genes involved in adipogenesis and lipolysis, as well as genes associated with energy homeostasis.

Materials and methods

Preparation of ID1216

Polygonatum sibiricum was authenticated by the National Agrobiodiversity Center (Korea) where the voucher specimen (29224-B99) was deposited. Steamed and dried *P. sibiricum* rhizomes (1 kg) purchased from HMAX Co., Ltd in 2011 (Jecheon, Korea) were mixed with 80% ethanol and extracted at 80 °C for 4 h in a reflux apparatus. After filtration, the residues were extracted again for 2 h. The combined extracts were concentrated under reduced pressure in a rotary vacuum evaporator to give a powdered extract (yield, 20.3%), which was designated ID1216.

Cell cultures and treatments

3T3L-1 and human embryonic kidney 293 (HEK293) cells were obtained from the Korean Cell Bank (Seoul, Korea), whereas C2C12 cells were purchased from the American Type Culture Collection (Manassas, VA). Cells were cultured in Dulbecco's Modified Eagles Medium (DMEM) with 10% fetal bovine serum (FBS) and antibiotics at 37 °C under 5% CO_2. Cells were treated with either ID1216 dissolved in dimethyl sulfoxide (DMSO) or DMSO alone (the control).

Two days after 3T3-L1 preadipocytes reached confluence (designated day 0), cells were treated with medium containing 1.7 μM insulin, 0.5 mM 1-methyl–3-isobutylxanthine, and 1 μM dexamethasone to induce differentiation and with 50 μg/ml ID1216 or not. The same concentration of the ID1216 was maintained when the medium was replaced. On day 3, the medium was replaced with progress medium (DMEM containing 10% FBS and 1.7 μM insulin), and the cells were cultured for 2 more days. Thereafter, fresh regular medium was substituted every day until day 12. Differentiation was monitored by morphological assessment and Oil Red O staining. For Oil Red O staining, cells were washed twice with phosphate buffered saline (PBS), fixed in 25% glutaraldehyde for 1 h, and stained for 30 min with 0.2% (w/v) Oil Red O solution in 60% (v/v) isopropanol. Cells were then washed several times with water and observed by microscopy (Nikon, Tokyo, Japan). To determine the extent of adipose conversion, triglycerides in differentiated cells treated with or without ID1216 were extracted, measured using a Triglyceride Colorimetric Assay Kit (Cayman Chemical, Ann Arbor, MI), and normalized to total cellular proteins.

C2C12 cells were plated in 6-well plates and grown to 100% confluence. To induce differentiation, the medium was replaced with DMEM containing 2% horse serum every day until day 3. Subsequently, cells were treated with varying doses of ID1216 from 6.2 μg/ml to 100 μg/ml to measure sirtuin-1 (SIRT1) protein expression and mRNA peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) for 1 h and 30 h, respectively.

Animal model

All animal procedures were in accordance with the guidelines issued by the Institutional Animal Care and Use Committee of the ILDONG Pharmaceutical Co., Ltd. (Protocol no. A1109-3). Male C57BL/6 J mice purchased from Central Lab Animal Inc. (Seoul, Korea) were housed 1 per cage and maintained in a constant temperature (25 °C) environment with a humidity of 50–60% under 12 h light-dark cycles. Animals were randomly divided into the following five groups ($n = 8$ per group): normal diet (ND), high-fat (HF) diet with PBS, 24 mg/kg orlistat, 250 mg/kg ID1216, and 1000 mg/kg ID1216. All treatments were administered by oral gavage every day for 10 weeks. The ND and HF groups were fed PicoLab Rodent Diet 20 (PicoLab, St. Louis, MO) and a high-fat diet (Research Diets, New Brunswick, NJ), respectively. The animals were given free access to food and water during the entire experimental period. Body weight was measured weekly, and food intake was recorded twice per week during the course of the study. At the end of the experiments, all mice were sacrificed and tissue weights were measured.

Measurement of fecal lipid contents

Feces from mice fed an HF diet with or without ID1216 for 2 weeks were collected for 3 d. To analyze fecal lipid levels, 0.5 g of each sample was extracted in chloroform:methanol (2:1, v/v), as described previously (Folch et al., 1957). The triglyceride content was quantified enzymatically using a Triglyceride Colorimetric Assay Kit.

Cold room test

Diet-induced obese mice fed an HF diet with or without drugs for 8 weeks were placed at 4 °C without food for 8 h. Rectal temperatures were taken every 2 h using a TH-8 Thermalert Monitoring Thermometer (Physitemp Instruments Inc., Clifton, NJ).

Oral glucose tolerance test

After mice were fasted for 8 h, glucose was administered orally (1.5 g/kg body weight). Blood samples were collected from mouse tails at 0, 30, 60, and 120 min after glucose loading, and blood glucose levels were measured using Accu-Chek Active (Roche, Basel, Switzerland). The area under the curve (AUC) over time was calculated.

Blood analysis

Blood samples were collected in heparinized tubes, separated by centrifugation, and stored at −20 °C until assayed. The concentrations of serum total cholesterol, as well as

high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), were determined using an EnzyChrom™ HDL and LDL/VLDL Assay Kit (Bioassay System, Hayward, CA). Serum glucose levels were measured using Accu-Chek Active, and serum triglycerides and free fatty acids were quantified by the Triglyceride Colorimetric Assay Kit and LabAssay™ NEFA (Wako, Richmond, VA), respectively.

Quantitative reverse transcription-polymerase chain reaction

Total RNA was prepared from homogenized tissues and cells using TRIsure (Bioline, London, UK). For quantitative reverse transcription-polymerase chain reaction (RT-PCR), cDNA was reverse transcribed from 2 μg of total RNA with the use of M-MLV reverse transcriptase and oligomers (Promega, Madison, WI). The resulting cDNA fragments were then amplified using IQ™ SYBR® Green Supermix (Bio-Rad, Hercules, CA) and the Peltier Thermal Cycler, according to the manufacturer's instructions (Bio-Rad, Foster City, CA), with the following primers: SIRT1, 5′-TGACTTCAGATCAAGAGATGGTATTTATG-3′ (forward) and 5′-TGGCTTGAGGATCTGGGAGAT-3′ (reverse); peroxisome proliferator-activated receptor gamma (PPARγ), 5′-ATCTTAACTGCCGGATCCAC-3′ (forward) and 5′-TG GTGATTTGTCCGTTGTCT-3′ (reverse); fatty acid synthase (FAS), 5′-CCTGCTATCATCTGACTTCCTCT-3′ (forward) and 5′-AGGGTGGTTGTTAGAAAGATCAA-3′ (reverse); lipoprotein lipase (LPL), 5′-AGGACACTTGTCATCTCA TTCCT-3′ (forward) and 5′-AGACATCTACAAAATCAG CGTCA-3′ (reverse); CCAAT/enhancer binding protein alpha (C/EBP1α), 5′-AGCTTACAACAGGCCAGGTTTCC-3′ (forward) and 5′-ACTCAGCTTTCTGGTCTGACTGG-3′ (reverse); sterol regulatory element-binding protein-1 c (SREBP-1 c), 5′-GCGCCATGGACGAGCTG-3′ (forward) and 5′-TTGGCACCTGGGCTGCT-3′ (reverse); PGC1α, 5′-CGATGTGTCGCCTTCTTGCT-3′ (forward) and 5′-CG AGAGCGCATCCTTTGG-3′ (reverse); and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), 5′-AGACTCCACG ACATACTCAGCAC-3′ (forward) and 5′-ACGGCAAATTC AACGGCACAG-3′ (reverse). The thermal cycling conditions were as follows: initial activation of DNA polymerase at 95 °C for 3 min, followed by 50 cycles of 95 °C for 15 s, 60 °C for 20 s, and 72 °C for 30 s. The expression data were normalized to GAPDH.

Histochemistry

Mouse tissues were fixed with formalin, dehydrated with ethanol, embedded in paraffin, and cut at 4 μm in thickness. Sections of epididymal adipose tissues and livers were subjected to hematoxylin and eosin staining and then analyzed by microscopy.

Immunoblotting

Total proteins were extracted in TEN buffer (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 2 mM ethylenediaminetetraacetic acid, 1% Triton X-100, 1 mM Na_3VO_4, protease inhibitor cocktail), and the protein concentration was determined by the bicinchoninic acid (BCA) assay. Proteins were electrophoresed on sodium dodecyl sulfate (SDS)-polyacrylamide gels after boiling for 5 min in SDS sample buffer and then blotted onto polyvinylidene fluoride membranes (Millipore, Bedford, MA). After blocking with Tris-buffered saline (TBS) (10 mM Tris-HCl, pH 7.4, 150 mM NaCl) containing 0.1% Tween 20 and 5% non-fat dried milk, membranes were probed overnight at 4 °C with the following primary antibodies diluted in blocking buffer: anti-SIRT1 antibody, anti-α-actin antibody (Cell Signaling Technology, Beverly, MA), anti-PGC-1α antibody (Santa Cruz Biotechnology, Santa Cruz, CA), or anti-β-tubulin antibody (Abcam, Cambridge, UK). Membranes were then washed and incubated with the appropriate secondary antibodies (Thermo Scientific, Rockford, IL) for 1 h at room temperature, followed by more washes. Finally, proteins were detected using a WEST-one™ Western Blot Detection System (iNtRON Biotechnology, Sungnam, Korea).

Statistical analysis

Data are presented as means ± the standard error of the mean. Statistical analysis was performed by one-way ANOVA using Sigma Stat 2.0 (San Rafael, CA), where $p < 0.05$ was considered significant for all comparisons.

Results

ID1216 reduces the body weight and tissue mass of HF diet-fed mice

To determine the impact of ID1216 treatment on HF diet-fed mice, we measured the body weight and food intake of the animals for 8 weeks. The results showed that ID1216 significantly reduced the final body weight (11.1–15.7%) and body weight gain (26.7–40.7%) of HF diet-fed mice (Table 1), compared with the vehicle-treated group. Despite there being little difference between the amounts of food consumed by the two groups over the study period, the food efficiency ratio was significantly lower in the ID1216-treated mice than the vehicle-treated controls. In addition, ID1216 significantly decreased the weight of epididymal adipose tissues and livers (Figure 1A), as well as the size of adipocytes, from the ID1216-treated mice (Figure 1B). Notably, HF diet-induced lipid accumulation in the liver tissues was also alleviated by ID1216 treatment (Figure 1A and B).

After ID1216 administration, we also monitored the mortality and clinical findings daily for 10 weeks. Autopsy of the mice revealed no gross abnormalities. Moreover, we investigated the safety of ID1216 by administrating either a single dose or repeated doses to Sprague–Dawley rats in a Good Laboratory Protocol-certified lab. The No-Observed-Adverse-Effect-Level of ID1216 was found to be over 2000 mg/kg (data not shown). These results suggest that ID1216 may have an impact on the body weight and tissue mass of the animals without toxicity or side effects.

ID1216 enhances the energy expenditure of HF diet-fed mice

The critical parameters that contribute to body weight maintenance include caloric intake and energy homeostasis.

Table 1. Effects of ID1216 on body weight, food intake, fecal triglyceride, and plasma lipids in mice fed a HF diet.

				ID1216	
	ND	HF	Orlistat	250 mg/kg	1000 mg/kg
Final body weight (g)	26.5 ± 0.6**	35.0 ± 1.5	30.5 ± 1.5**	29.9 ± 1.4**	31.4 ± 1.1
Body weight gain (g)	5.0 ± 0.3**	13.5 ± 1.1	8.9 ± 0.6**	8.4 ± 0.6**	9.8 ± 0.7
Daily food intake (g/d)	4.4 ± 0.1**	3.0 ± 0.0	2.9 ± 0.1	2.8 ± 0.1**	2.8 ± 0.1**
Food intake efficiency ratio	0.02 ± 0.00*	0.08 ± 0.01	0.06 ± 0.00*	0.05 ± 0.00**	0.06 ± 0.00
Fecal triglyceride (mg/g dry feces)	1.2 ± 0.2**	2.9 ± 0.2	11.6 ± 0.5**	1.4 ± 0.1**	1.2 ± 0.1**
LDL (mg/dl)	38.3 ± 1.8*	57.5 ± 2.3	47.1 ± 1.1	42.0 ± 1.4*	40.5 ± 0.6*
HDL (mg/dl)	75.9 ± 0.9*	99.6 ± 3.2	95.9 ± 1.2	88.0 ± 3.0	90.9 ± 0.7
Total cholesterol (mg/dl)	97.9 ± 7.2*	167.4 ± 42.2	173.3 ± 18.3	144.0 ± 36.1	151.3 ± 12.2
Triglyceride (mg/dl)	107.7 ± 3.4	122.9 ± 2.6	112.1 ± 1.4	90.5 ± 1.5*	90.1 ± 1.8*
Free fatty acids (mEq/l)	1.2 ± 0.02	1.3 ± 0.03	1.2 ± 0.01	1.0 ± 0.01**	0.9 ± 0.01**

Data are expressed as means ± SEM of 8 mice per group. *$p < 0.05$, **$p < 0.01$ versus HF. Food efficiency ratio = body weight gain for experimental period (g)/food intake for experimental period (g). Feces were collected twice for 3 d during the period of the experiment. Fecal triglyceride content was measured after 2 weeks. Data are expressed as means ± SEM of 6 mice per group. *$p < 0.05$, **$p < 0.01$ versus HF. Blood samples of mice fed a HF diet were collected after 10 weeks. Data are expressed as means ± SEM of 8 mice per group. *$p < 0.05$, **$p < 0.01$ versus HF.

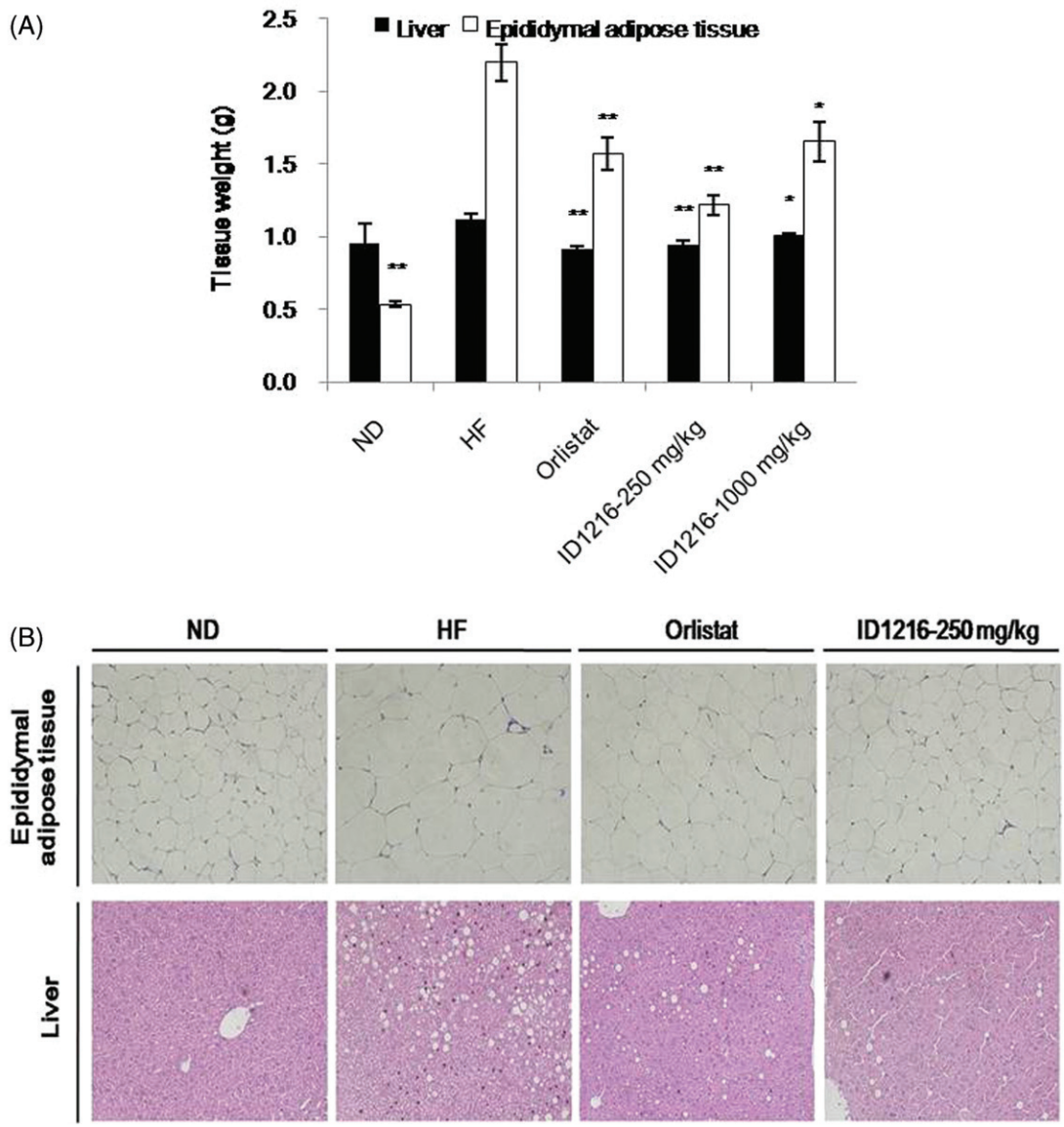

Figure 1. Effects of ID1216 on the adipose tissue and liver. Mice fed an HF diet for 10 weeks were sacrificed, and epididymal adipose tissue and liver weights were measured (A). Data are expressed as means ± SEM of 8 mice per group. *$p < 0.05$, **$p < 0.01$ versus HF. Sections of epididymal adipose tissues and livers were subjected to hematoxylin and eosin (H&E) staining and then analyzed by microscopy (B). H&E were stained sections of epididymal adipose tissues and liver from each group showing the various degrees of lipid droplets formation.

Since the food efficiency ratio and adipose tissue weight were lowered in the ID1216-treated mice, the reduced body weight gain may not be caused by cumulative caloric intake. Several herbal extracts have been reported to inhibit pancreatic lipase activity and prevent weight gain (de la Garza et al., 2011). In our present study, the fecal triglyceride content in the orlistat-treated group was found to be 4-fold higher than that in the vehicle-treated group, whereas the triglyceride level in the ID1216-treated mice was 2.4-fold lower than that in the control animals (Table 1). These results indicate that ID1216 did not inhibit pancreatic lipase activity in the HF diet-fed mice. Next, we performed a cold test to examine the effect of ID1216 administration on the regulation of body temperature (Figure 2A). The mice fed an HF diet with or without ID1216 for 7 weeks were exposed to a cold environment for 8 h, and the changes in core body temperature were monitored every 2 h by measuring rectal temperatures. We observed that ID1216 treatment (1000 mg/kg) led to a 6.2% decrease from the initial temperature, whereas the vehicle-treated mice experienced a 9.8% temperature decrease. These results suggest that enhanced energy expenditure by ID1216 treatment may play some important role in body weight reduction.

ID1216 improves HF diet-induced metabolic disorders

To determine whether ID1216 affects metabolic alterations in HF diet-fed mice, we compared the plasma concentrations of triglycerides, total cholesterol, HDL-C, LDL-C, and free fatty acids in diet-induced obese mice treated with or without ID1216 (Table 1). We found that ID1216 treatment improved the serum lipid profile of the animals. In particular, compared with the vehicle-treated group treatment with 1000 mg/kg ID1216 led to significant decreases in the serum levels of LDL-C, triglycerides, and free fatty acids (29.6, 26.7, and 30.8%, respectively). Moreover, because obesity-induced lipid accumulation and alterations in fatty acid composition in various tissues are considered to be a cause of insulin resistance, we performed oral glucose tolerance test analysis after 9 weeks of treatment with or without ID1216 (Figure 2B and C). Our results showed that ID1216 treatment not only ameliorated HF diet-induced hyperglycemia in a

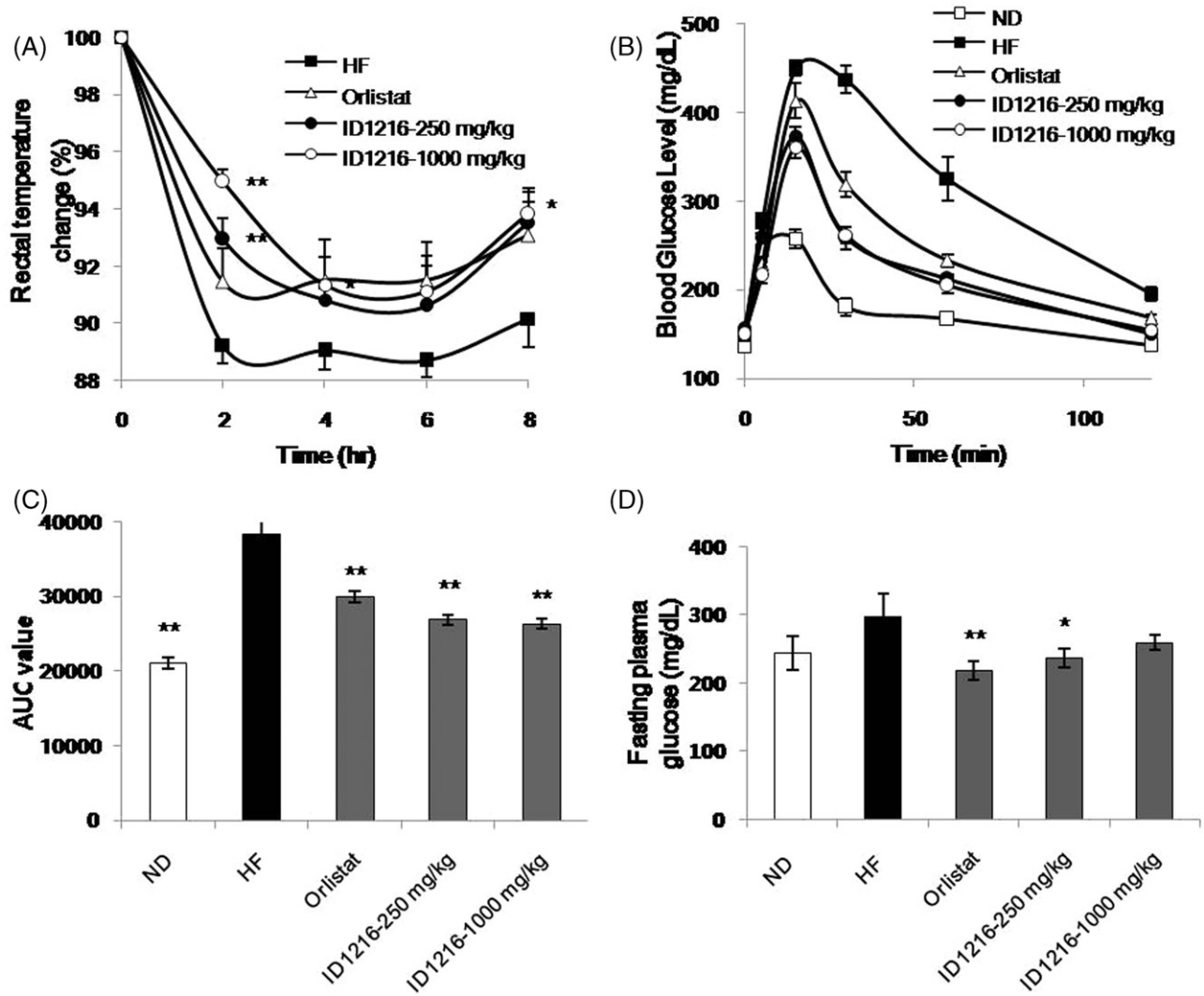

Figure 2. Effects of ID1216 on adaptive thermogenesis and glucose tolerance in diet-induced obese mice. Mice fed an HF diet for 7 weeks were exposed to 4 °C and rectal temperature was monitored for 8 h (A). Data are expressed as means ± SEM of 6 mice per group. *$p < 0.05$, **$p < 0.01$ versus HF. Blood glucose concentrations (B) and AUC (C) during the OGTT and the fasting plasma glucose (D). Data are expressed as mean ± SEM of 8 mice per group. *$p < 0.05$, **$p < 0.01$ versus HF.

dose-dependent manner but also significantly decreased fasting plasma glucose levels (Figure 2D).

ID1216 regulates adipocyte differentiation and energy homeostasis

To investigate the effect of ID1216 on the modulation of adipocyte differentiation, 3T3-L1 cells were treated with ID1216 during differentiation and then subjected to Oil Red O staining and triglyceride measurements (Figure 3). The accumulation of intracellular fat was significantly reduced by ID1216 treatment, compared with the vehicle control, suggesting that ID1216 may efficiently block the differentiation of 3T3-L1 cells into adipocytes. We also evaluated the expression of genes that are closely associated with adipogenesis by quantitative RT-PCR (Table 2). The results indicated that the mRNA expression levels of SREBP-1 c, PPARγ, C/EBPα, LPL, and FAS were significantly lower in the ID1216-treated group than the vehicle-treated group. In contrast, the mRNA levels of SIRT1, which plays an important role in thermogenesis, were elevated by ID1216 treatment at the early stage of adipogenesis.

SIRT1 is the master regulator of mitochondrial biogenesis and regulates the expression of PGC-1α, which modulates energy homeostasis in response to environmental and nutritional stimuli (Picard et al., 2004; Tzameli et al., 2004). Therefore, we examined the effect of ID1216 on the protein expression of SIRT1 in C2C12 myocytes and HEK293 cells (Figure 4A and B). We observed that ID1216 treatment not only increased SIRT1 protein expression in a dose-dependent manner but also induced PGC-1α mRNA expression in C2C12 myocytes (Figure 4C).

Discussion

Some human and animal studies have reported the beneficial effects of the dried rhizome of *P. sibiricum* and its extracts against aging, metabolic problems, infection, and neuronal cell death (Chen et al., 1993; Kato & Miura, 1993; Kato et al., 1993; Kim et al., 2007; Miura & Kato, 1995; Miura et al., 1995). Despite these studies, the pharmacological action mechanisms of *P. sibiricum* on obesity and related metabolic disorders have not been yet thoroughly investigated.

In the present study, we demonstrated that ID1216 not only prevented body weight gain but also improved plasma lipid profiles and glucose tolerance in HF diet-induced obese mice. To the best of our knowledge, this study is the first to investigate the effects of an alcohol extract of dried *P. sibiricum* rhizomes on obesity and related metabolic disorders induced by an HF diet in mice. It is also noteworthy that ID1216 did not affect the suppression of food intake or the inhibition of pancreatic lipase activity. These results suggest that ID1216 may lead to the prevention of body weight gain by other actions rather than the inhibition of food intake or intestinal fat absorption. Based on these findings, we hypothesize that ID1216 may control HF diet-induced body weight gain in mice by modulating adipogenesis and enhancing energy expenditure.

Adipogenesis is the process which preadipocytes differentiate into mature fat-accumulating adipocytes and plays an important role in both the development of obesity and against energy homeostasis (Ali et al., 2013). Our results show that ID1216 reduced the size of adipocytes in mice and inhibited

Table 2. Effect of ID1216 on the expression of key transcription factors during 3T3-L1 adipocyte differentiation.

		Differentiated day		
Gene	Drug	1	2	3
SIRT1	Vehicle	1.00 ± 0.06	1.22 ± 0.05	0.77 ± 0.08
	ID1216	1.28 ± 0.03**	1.16 ± 0.15	0.63 ± 0.04
PPARγ	Vehicle	1.00 ± 0.06	2.04 ± 0.21	3.53 ± 0.42
	ID1216	1.02 ± 0.03	2.26 ± 0.08	2.26 ± 0.17*
FAS	Vehicle	1.00 ± 0.13	1.49 ± 0.06	1.80 ± 0.17
	ID1216	0.86 ± 0.15	1.53 ± 0.10	1.21 ± 0.10*
LPL	Vehicle	1.00 ± 0.11	4.46 ± 0.34	6.34 ± 0.48
	ID1216	0.97 ± 0.01	3.37 ± 0.07*	4.90 ± 0.60*
C/EBP1α	Vehicle	1.00 ± 0.07	5.38 ± 0.25	8.72 ± 0.25
	ID1216	1.04 ± 0.05	4.96 ± 0.51	5.91 ± 0.47**
SREBP-1 c	Vehicle	1.00 ± 0.12	2.33 ± 0.10	2.29 ± 0.09
	ID1216	0.85 ± 0.11	2.02 ± 0.02*	1.84 ± 0.09**

The differentiating 3T3-L1 cells were treated with ID1216 (50 μg/ml) and RNAs were isolated at various periods (from 1 to 3 d). Relative mRNA levels were analyzed by quantitative RT-PCR. The values indicate relative mRNA levels compared with the vehicle group. Data are means ± SEM ($n = 3$). *$p < 0.05$, **$p < 0.01$ versus the vehicle group.

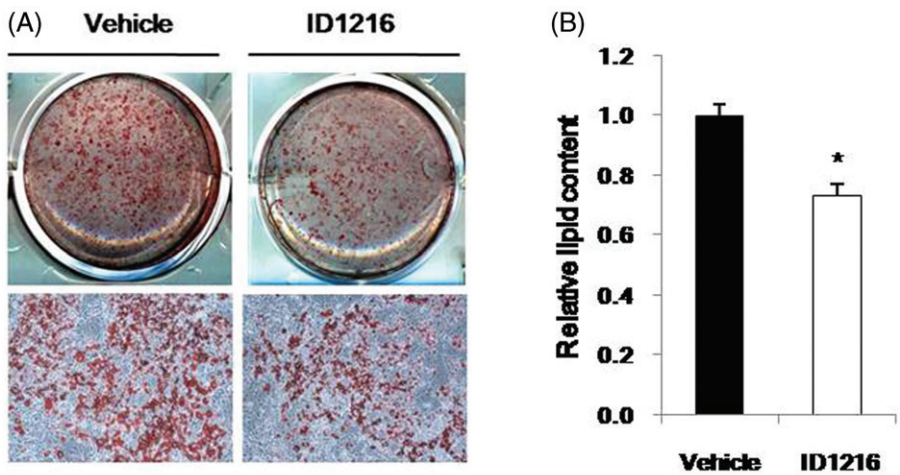

Figure 3. Inhibition of adipocyte differentiation by ID1216. 3T3-L1 cells were differentiated for 14 d with or without ID1216 (50 μg/ml). Oil red O-stained dishes and high magnification (× 200) of cells (A) and triglyceride normalized to total cellular protein (B) were observed the content of lipid accumulation from each group. Data are expressed as means ± SEM ($n = 3$). *$p < 0.05$ versus the vehicle group.

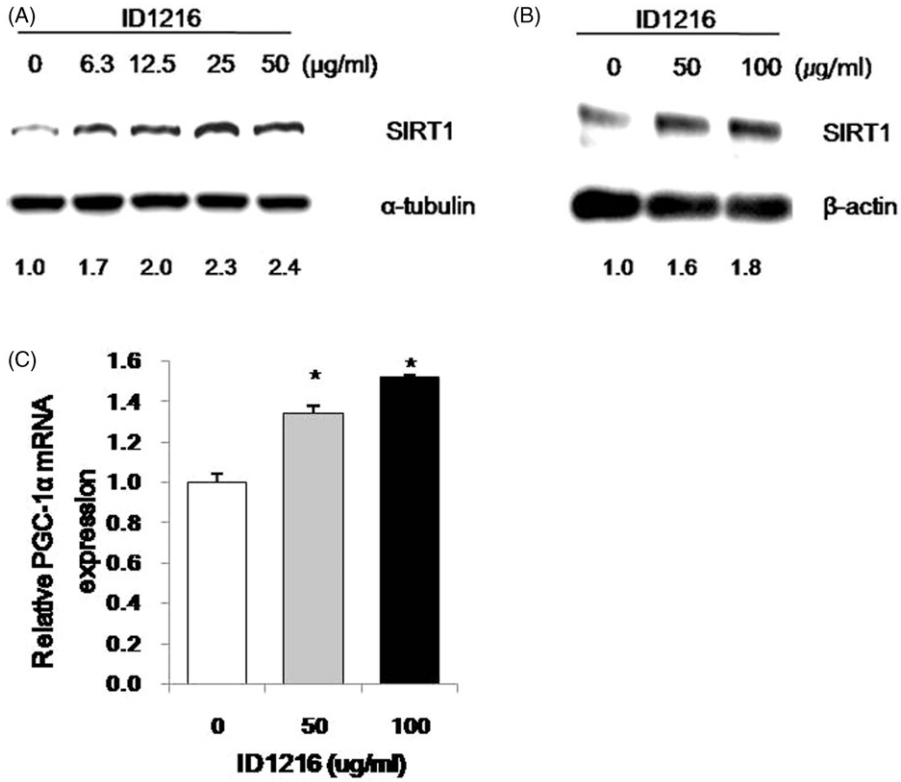

Figure 4. Effects of ID1216 on the expression of SIRT1 and PGC-1α. HEK293 cells (A) or C2C12 myocytes (B) were treated with various concentrations of ID1216 and extracted proteins after the mentioned time, respectively. The protein expression level of SIRT1 was detected by Western blot analysis and the relative ratio is shown. C2C12 myocytes were treated with or without ID1216 for 30 h and relative mRNA level was analyzed by quantitative RT-PCR (C). The values indicated relative mRNA levels compared with the vehicle group. Data are means ± SEM ($n = 3$). *$p < 0.05$, **$p < 0.01$ versus the non-treated group.

the differentiation of 3T3-L1 adipocytes. ID1216 also suppressed the mRNA expression of PPARγ, C/EBPα, LPL, and FAS sequentially during adipogenesis. PPARγ is a nuclear receptor critical for adipocyte differentiation and the maintenance of mature adipocytes through its interaction with the co-activator C/EBPα, and the interaction subsequently regulates the transcription of both LPL and FAS. Therefore, we speculate that the inhibitory effects of ID1216 on fat storage and adipogenesis may result from the repression of genes that are involved in adipogenesis and fat storage, such as PPARγ, C/EBPα, LPL, and FAS.

Many studies have identified SIRT1 as an important regulator of metabolic process, including adipogenesis and adipolysis (Jin et al., 2010; Nogueiras et al., 2012; Picard et al., 2004; Qiang et al., 2007; Tzameli et al., 2004). Theses previous studies suggested that the up-regulation of SIRT1 in mice and human adipose tissues suppressed adipogenesis and induced lipolysis (Kelly, 2006; Picard et al., 2004). Resveratrol, a SIRT1 activator, is a well-known potential therapy for insulin resistance-related conditions, such as type 2 diabetes mellitus and metabolic syndrome, and exerts antiaging effects by mimicking calorie restriction (Milne et al., 2007). Resveratrol has been shown to decrease lipid accumulation by repressing PPARγ with SIRT1 activation and inhibiting the expression of SREBP-1 c, which is involved in the modulation of lipogenic genes such as FAS (Fullerton & Steinberg, 2010; Wang et al., 2009b). Similarly, in this study, ID1216 suppressed the mRNA expression of PPARγ, SREBP-1 c, and FAS and induced SIRT1 expression during 3T3-L1 adipogenesis. Although further studies are needed, this result suggests that ID1216 treatment may repress adipogenic genes through the up-regulation of SIRT1 and consequently lead to the prevention of body weight gain and metabolic disorders induced by an HF diet.

SIRT1 also regulated PGC-1α, which induces mitochondrial biogenesis and respiration by interacting with transcription factors, such as estrogen-related receptor-α, nuclear respiratory factor-1 and -2, PPARα and δ, and thyroid hormone receptor (Lagouge et al., 2006). In brown fat, PGC-1α stimulates adaptive thermogenesis by up-regulating the expression of uncoupling protein-1 (Du et al., 2013). PGC-1α has also been suggested to contribute to the improvement of insulin resistance through muscle fiber type switching (Mootha et al., 2003, 2004). Previously, SIRT1 was reported to induce both the expression and the activity of PGC-1α (Nemoto et al., 2005). In this study, we show that ID1216 treatment increased the expression level of PGC-1α and SIRT1 in C2C12 myocytes. In addition, ID1216-treated mice had a higher core body temperature than vehicle-treated animals when exposed to an acute cold environment. Lagouge et al. (2006) reported that resveratrol-treated mice maintained their body temperature higher as compared with that of vehicle-treated mice under cold conditions. The authors suggested that resveratrol induced PGC-1α expression and mitochondrial amplification in brown adipose tissue and subsequently stimulated adaptive thermogenesis and prevented body weight gain. In our studies, we also observed that ID1216 treatment comparably increases PGC-1α expression and enhances adaptive thermogenesis.

Although ID1216 has shown the antiobesity effect in this study, the bioactive component in ID1216 remains to be elucidated. Recently, homoisoflavanones and isoflavones besides steroidal saponins, furostanol glycoside, and dipeptide have been identified from *P. odoratum* (Wang et al., 2009a, 2013). It had been reported that the isoflavone derivatives

showed differential effects on the activation and expression of SIRT1 and activator of PGC-1α (Rasbach & Schnellmann, 2008) and homoisoflavonoids had glucose uptake-stimulatory activity in 3T3-L1 adipocytes (Zhang et al., 2010). ID1216 as an alcohol extract of dried *P. sibiricum* rhizomes might be also a rich source of flavonoids. Although it has not yet been clarified whether these flavonoids have a preventive effect on obesity *in vivo*, we assumed that flavonoids from ID1216 may be responsible for the observed physiologic effects.

Conclusions

Taken together, our results suggest that increased SIRT1 expression levels induced by ID1216 result in PGC-1α activation and PPARγ repression, thereby leading to the inhibition of metabolic disorders in diet-induced obese mice. Considering efficacy and safety of ID1216 elucidated in our studies, we believe that it is worth developing ID1216 as an alternative for the prevention and alleviation of diet-induced obesity. Further chemical and pharmacological investigation should be carried out to evaluate the mechanism of regulating the SIRT1-PGC1α pathway.

Declaration of interest

The authors report no declarations of interest.

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